

Application No.: 10/606,100  
Amendment Dated: January 4, 2008  
Office Action dated February 27, 2006  
Attorney Docket No.: 2233.001

**RECEIVED  
CENTRAL FAX CENTER**

**JAN 04 2008**

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listing of claims in the application.

**Listing of Claims:**

**CLAIMS**

1. (currently amended) A method for automatic alignment of tilt series in an electron microscope, comprising:
  - applying markers to a sample to be imaged by the electron microscope;
  - providing a tilt series of images of the sample;
  - identifying a first set of candidate markers in each of the images in the tilt series;
  - attributing at least one probability parameter to each candidate marker in each image;
  - characterized in that the method further comprises:
    - selecting a second set as a subset of candidate markers from the first set of candidate markers on the basis of said at least one probability parameter;
    - projecting the candidate markers in the second set onto a sole image;
    - applying a fitting algorithm to determine a set of parallel straight lines or very elongate ellipses best fitting the candidate markers in the sole image to identify a third subset of candidate markers;
    - aligning the images in the tilt series on the basis of the third subset of identified candidate markers.
2. (original) A method according to Claim 1 in which the fitting algorithm used to determine the set of parallel straight lines comprises the Hough transformation.

Application No.: 10/606,100  
Amendment Dated: January 4, 2008  
Office Action dated February 27, 2006  
Attorney Docket No.: 2233.001

3. (original) A method according to Claim 1 in which the fitting algorithm used to determine the set of parallel straight lines or to determine a set of very elongate ellipses is constituted by the Generalized Hough transformation.

4. (original) A method according to Claim 1 in which, before identifying candidate markers in each of the images in the tilt series, a cross correlation process is applied to the images of the tilt series.

5. (previously presented) A method according to Claim 1 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

6. (currently amended) A method according to ~~Claim 2~~ for automatic alignment of tilt series in an electron microscope, comprising:

applying markers to a sample to be imaged by the electron microscope;

providing a tilt series of images of the sample;

identifying a first set of candidate markers in each of the images in the tilt series;

attributing at least one probability parameter to each candidate marker in each image;

characterized in that the method further comprises:

selecting a second set as a subset of candidate markers from the first set of candidate markers on the basis of said at least one probability parameter;

projecting the candidate markers in the second set onto a sole image;

applying a fitting algorithm to determine a set of parallel straight lines or very elongate ellipses best fitting the candidate markers in the sole image to identify a third subset of candidate markers, in which the fitting algorithm further comprises: including;

Application No.: 10/606,100  
Amendment Dated: January 4, 2008  
Office Action dated February 27, 2006  
Attorney Docket No.: 2233.001

deriving for each candidate marker in the second set a sine-shaped curve based on the coordinates of the corresponding candidate marker, according to the Hough transformation;

deriving from the sine-shaped curves a number of histograms indicating, for each direction, the relation between the density of candidate markers and the line distance parameter according to the Hough transformation;

applying an entropy operation to each of the histograms, resulting in a set of entropy parameters, one entropy parameter for each histogram;

establishing the minimum value in the set of entropy parameters;

identifying the histogram corresponding to said minimum value as the one showing the highest degree of peak diversity;

selecting from the latter histogram a number of peaks; and

deriving from each peak position in the histogram the corresponding line distance parameter according to the Hough transformation.

7. (previously presented) A method according to Claim 2 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

8. (previously presented) A method according to Claim 3 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

9. (previously presented) A method according to Claim 4 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.